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CHEMICAL ANALYSES OF PLANT TISSUES FROM THE HUBBARD BROOK ECOSYSTEM IN NEW HAMPSHIRE

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INTRODUCTION

DURING the past 7 years we have been involved in a multidisciplinary study of several small watershed-ecosystems in the Hubbard Brook Experimental Forest, West Thornton, New Hampshire. The Hubbard Brook Experimental Forest is maintained and operated by the U.S. Forest Service. A primary focus of these studies is to establish quantitative nutrient budgets for undisturbed and man-manipulated northern hardwood forest ecosystems (Bormann and Likens 1967).

Internal storage and cycling of nutrients by the vegetation is fundamental to understanding the functional cycling of nutrients in an ecosystem. Thus as a part of our ecosystem study, we have chemically analyzed plant tissues of most of the important plant species within the Hubbard Brook Ecosystem. The purpose of this paper is to present the collective results of these analyses and to describe and verify the procedures whereby they were obtained. Subsequently these data will be used in calculations of nutrient cycling and nutrient budgets for the Hubbard Brook Ecosystem. Tissue analysis has been used by many workers in an attempt to assess the nutritional status of plants (see e.g. Lundegårah 1945, Goodall and Gregory 1947, Bould *et al,* 196o, Smith 1962, Stone 1968). However, the use of tissue analysis to characterize nutritional status of plants requires well-designed sampling procedures and careful interpretation of results. The nutritional or physiological implications of data collected in this study will not be evaluated here.

The Hubbard Brook Experimental Forest ranges in altitude from 229 to 1006 m and covers 3076 ha of rugged terrain. The forest is characterized by uneven-aged, well-stocked, second-growth northern hardwoods with more coniferous species at higher elevations and on north facing slopes. The major overstory tree species are sugar maple, *Acer saccharum,* beech, *Fagus grandifolia,* yellow birch, *Betula alleghaniensis,* and red spruce, *Picea rubens}* with some white birch, *Betula papyrifera* and balsam fir, *Abies balsamea.* The Experimental Forest was extensively cut about 1919, but no cutting or fire has occurred since. A detailed description of the tree vegetation and herbaceous flora are given by Bormann *et al.* (1970) and Siccama *et al.* (1970).

Bouldery till covers most of the area and is generically similar to the bedrock lithologies, which are Littleton Formation sillimanite-zone gneiss and Kinsman quartz monzonite (Johnson *et al.* 1968). Based upon available nutrients, weathering rates and vegetation analysis, the area is considered to be relatively oligotrophic (Likens *et al.* 1967; Fisher *et al.* 1968; Johnson *et al.* 1968; Bormann *et al.* 1970; Siccama *et al.* 1970).

Six small, well-defined watersheds have been selected for intensive study in the Hubbard Brook Experimental Forest (Bormann and Likens 1967). These are all steep (average slope, 29%), southeast facing watersheds with similar vegetation, till and bedrock. These watershed-ecosystems range in size from 12 to 43 ha and in altitude from 500 to 800 m. The climate is basically humid continental with a short, cool summer and a long, cold winter. Additional details concerning the topography, climatology, geology, soils and biology are given in Likens *et al.* (1967) and U.S. Forest Service (1964).

Methods and Procedures

General Field Techniques

THE plants analyzed in this study were collected in, or adjacent to Watershed No.6. This watershed-ecosystem has been used extensively by us in an attempt to characterize nutrient cycling and energy flow in a small well-defined, undisturbed northern hardwood forest ecosystem. Watershed 6 was divided for purposes of this study into upper (724-785 m), middle (648-724 m) and lower (553-648 m) thirds to evaluate altitudinal effects on nutrient concentrations in plant tissues. Species nomenclature is according to Fernald (1950), except where authorities are cited.

Tissue samples were obtained from at least six individuals of different size for each tree species. Representative samples of the entire plant were collected, e.g. large numbers of leaves and branches were collected randomly from the entire crown. Samples of *Prunus pensylvanica* were collected during July and August 1967. All other samples were collected during July and August 1966. Average results for the species are presented here, and some mention is made of elevational differences.

The herbaceous flora was sampled during 1967 in 3 ways: I) individual plants (shoots, roots and bulbs) of the major spring ephemeral flora were collected during the last week of May 1967, 2) composite samples (dry weight of about 30 g) were obtained on 24-25 July 1967 for each of the major 29 species of herbaceous plants on each of the elevational subdivisions of Watershed 6, and 3) five replicate, composite samples were obtained for each of the 6 dominant herbaceous species on 7 September 1967 from the lower subdivision of Watershed 6. The composite samples were obtained by combining shoots from all plants collected during a 3o-minute random search on each subdivision.

We have attempted to maintain very high quality control on our plant tissue analysis and have taken special precautions to avoid the various sources of potential contamination, particularly during collection of the samples. Plastic gloves were used when collecting or handling samples. As soon as the samples of plant material were collected, they were placed in clean paper bags and promptly returned to the laboratory. The samples were then removed from the paper bags and either processed immediately or placed in clean plastic bags and frozen on the same day as they were collected.

General Laboratory Techniques

All the water used' in these analyses was prepared by passing tap water through an Ilico-Way Ion Xchange column (research model).l This water was essentially free of ions; the electrical conductivity ranged between 0.7 and r.o μmhos / cm^2 at 20°C. The deionized water was stored in polyethylene bottles.

Plastic gloves were worn at all times when handling plant materials, glassware and crucibles in the laboratory.

All glassware for sample solutions, reagents, etc. was cleaned by soaking (minimum of 20 minutes) in concentrated HNO_3, and then was rinsed three times with deionized water. New 4 oz. (113 g) glass jars with screw caps were vacuumed thoroughly before they were used to store dried plant tissue. All polyethylene containers were washed in a 50% solution of HC1, rinsed once with tap water and then three times with deionized water. Cheesecloth bags were rinsed several times in deionized water, and dried before use.

Plant tissues were ground in a Wiley Mill with a 20 mesh stainless steel screen. The mill and screen were vacuumed thoroughly before use and between each sample. Small particles or other residue were dislodged from the interior of the mill after each sample with a camel's hair brush and metal probe.

Preparation, Drying and Grinding of Samples

Foliage samples were not washed prior to analysis. Guha and Mitchell (1965) reported that out of 21 elements measured in beech foliage only Fe, V, Ti, Al and Cr were appreciably reduced by washing. Stone (1968) also suggested that washing may be very critical for iron analyses to eliminate contamination, particularly if soil dust is prevalent. We believe that soil dust is a very small component of the meteorologic chemical input to the Hubbard Brook Experimental Forest (Likens *et al.* 1967); however, we have not made specific measurements for iron in this regard.

Leaves and current twigs of trees, and shoots of herbaceous plants were placed in washed cheesecloth bags. The bags were closed with rubber bands, and the sample was dried in a forced draft oven at 80°C for about 48 hours. Most of the dried samples were ground in the Wiley Mill, but small volume samples were broken or cut into very small pieces with clean stainless steel scissors.

1. Use of trade names herein is solely for identification and does not necessarily imply endorsement by the authors or their agencies.

Root and bark samples from trees were selected to avoid excessive encrustations of lichens, algae, etc. If these materials were present, they were carefully scraped off. The root and bark samples then were washed in a detergent solution (1% Alconox), and rinsed three times in deionized water. Depending upon the size of the sample, it was cut into strips or small pieces, either with scissors or a clean metal chisel, before grinding in the Wiley Mill. Edges or ends of samples that were originally exposed in the field were trimmed off and discarded. Samples then were bagged, dried and ground.

Samples of wood from trees were cut from cross-sectional discs obtained from the bole of the tree. We used a tilting arbor saw with a clean 10" (25.4 cm) cut-off blade to trim away the edges of the disc that had been in contact with the chain saw in the field. The wood samples were cut into small pieces with a clean chisel, placed in cheesecloth bags, and dried at 80°C for 48 hours or until thoroughly dry. They were then ground in a Wiley Mill.

After grinding, each sample was 1) placed in a clean 4 oz. (113 g) glass screw top bottle, 2) dried for 72 hours in a forced draft oven at 80°C and 3) sealed in a bottle while warm. The samples were stored in a constant temperature room at a temperature of 2–5°C prior to chemical analysis.

Chemical Analysis of Plant Tissues

Samples removed from the constant temperature room were placed in the drying oven (caps removed) at 80°C for 48 hours. About 2 g were taken from the thoroughly mixed sample and 1) placed in a clean, dried (500°C for a minimum of 30 minutes), tared, fused silica crucible, 2) weighed to the nearest 0.1 mg on an electronic balance to obtain sample dry weight, and 3) placed in a cold muffle furnace. The temperature in the furnace was brought slowly to a maximum of 500±20°C, and allowed to remain at this temperature for a minimum of 2 hours. The oven door was cracked until the temperature dropped to 300°C and the crucibles were removed to a desiccator. Ash weight was obtained on the samples at room temperature.

About one ml deionized water was added to the ash in the crucible to reduce spattering. Then 10 ml of redistilled 6N HCl were added to the crucible. The entire contents were brought slowly to the boiling point on a hot plate, then cooled and filtered through Whatman #42 filter paper. The filter paper had been washed previously with 6N HCl and deionized water. The crucibles and filter paper were rinsed three times with deionized water to facilitate a quantitative transfer of the sample. The filtrate was collected in a clean volumetric flask and brought to volume with deionized water. The filter papers and any

residue were ashed by the same procedure as above and weighed to obtain filter paper and residual ash weight.

Standard solutions were prepared for analysis on the Perkin Elmer Atomic Absorption Spectrophotometer, Model 303, from Certified Standards for atomic absorption spectrophotometry (Fisher Scientific Co.) and appropriate amounts of redistilled 6N HCl (to approximate the acidity of the sample). Ca^{++} and Mg^{++} standards were diluted appropriately and a "buffer" solution containing 2% La_2O_3 and 50% HCl was added in a ratio of 1 part buffer to 10 parts solution. All cation analyses were done by atomic absorption spectrophotometry.

The 1-amino-2-napthol-4-sulfonic acid method was used to measure ortho phosphate concentrations in the sample solutions. Measurements were made on a Spectronic 20 Colorimeter (Bausch & Lomb).

Since we used a dry ashing technique in these cation and phosphorus analyses, it was necessary to determine whether significant quantities of any element was rendered insoluble in the process. This frequently occurs when a sample contains appreciable amounts of silica. Cations may be bound by silica, perhaps partially adsorbed, partially absorbed, and partially occluded, and these silica "complexes" are essentially insoluble in dilute HCl, even with heating. Preliminary analyses, using an ashing temperature of 550°C, indicated that samples of tree leaf tissue collected in the autumn at Hubbard Brook contained considerable amounts of insoluble ash (silica), and spectrographic analysis of the insoluble ash showed that considerable amounts of manganese, with smaller amounts of zinc, calcium, magnesium, copper, iron and sodium were bound on the silica, which was retained by the filter paper. No phosphorus was detected. However, subsequent analyses indicated that a smaller amount of insoluble ash was found when the samples were ashed at a lower temperature (480–500°C).

Therefore, the insoluble ash retained by the filter, and the filter were ashed again at 500°C and the residual ash weighed. The silica in this residue was vaporized in platinum crucibles with hydrofluoric acid in the presence of sulfuric acid.[2] After removal of the silica, the residue was dissolved in dilute HCl, made to volume and analyzed.

Only those tree tissues that had an insoluble ash $>0.4\%$ of the sample dry weight were analyzed (Table 1). For these a composite sample of the insoluble ash for each tissue was analyzed and a proportional correction applied, if necessary, to the original value based on filtrate analysis. The proportional correction was based on the assumption that the amount of cation retained was

2. Hydroflouric acid treatment was done under contract at the University of New Hampshire Engineering Experiment Station, Durham.

proportional to the amount of insoluble ash for any given tissue and species. Corrections less than 3% were ignored.

The majority of the calculated "corrections" were negligible or very small (<3%). However some corrections were necessary particularly for copper and zinc, and for root and bark samples. The copper content of *Fagus* bark was increased about 3-fold, and was the largest correction made. The relatively high content of silica in the root samples (Table 1) may be some indication of contamination by soil particles even though the samples were carefully cleaned and washed.

TABLE 1. AMOUNT OF INSOLUBLE ASH, AS % OF SAMPLE DRY WEIGHT, IN TISSUES OF TREE SPECIES AT HUBBARD BROOK.

	Acer saccharum	Betula alleghaniensis	Fagus grandifolia	Acer spicatum	Picea rubens
Roots	1.52	0.73	1.44	0.68	0.41
Leaves	0.99	0.31	0.87	0.34	0.40
Bark	0.10	0.03	0.59	0.12	0.18
Branches	0.04	0.02	0.30	0.02	0.13
Current Twigs	0.06	0.07	0.14	0.05	0.05
Light Wood	0.009	0.004	0.032	0.003	0.009
Dark Wood	0.002	0.128	0.009	0.029	—

No chemical analyses were made for insoluble ash in the herbaceous samples because most of them contained relatively little insoluble ash. However, there was a great amount of variation both between and within species. Notably high average values were found in *Carex* spp. (3.2% of sample dry weight), *Dryopteris phegopteris* (3.1%), *Athyrium filix-femina* (2.4%), *Viola rotundifolia* (2.0%), *Dryopteris noveboracensis* (1.3%), *Galium triflorum* (1.1%), *Clintonia borealis* (0.8%), *Aster acuminatus* (0.7%), *Oxalis montana* (0.7%), *Dennstaedtia punctilobula* (0.6%) and *Lycopodium lucidulum* (0.4%). In sharp contrast to *V. rotundifolia, V. incognita* contained only 0.02% insoluble ash. It is assumed that the concentration of zinc and copper reported here may be somewhat low for those species with high insoluble ash contents.

Analyses for total nitrogen and sulfur were done at the Engineering Experiment Station of the University of New Hampshire. Nitrogen was determined according to the method covered in section 2.044, and sulfur by the methods covered in sections 6.059 and 6.060 of the Official Methods of the Association of Official Agricultural Chemists (1965, 10th edition).

Comparison with Reference Samples

One of the more difficult problems in the chemical analysis of biological material is to attain a good reference sample to check methods and standardize

TABLE 2. A COMPARISON OF OUR ANALYSES WITH REFERENCE VALUES FOR SAMPLES OF PLANT TISSUE. VALUES GIVEN ARE MEANS AND STANDARD ERRORS BASED UPON DRY WEIGHT ANALYSES.*

	Apple[1]	Cherry[1]	Citrus[1]	Peach[1]	Kale[2]
Calcium (sample %)					
our value	1.02 ± .05	2.54 ± .07	3.32 ± .23	1.78 ± .06	3.83 ± .05
reference value	1.17 ± .04	2.95 ± .07	3.86 ± .13	1.98 ± .04	4.14 ± .22
Magnesium (sample %)					
our value	0.30 ± .01	0.89 ± .03	0.29 ± .01	0.45 ± .01	0.164 ± .002
reference value	0.36 ± .003	0.92 ± .04	0.33 ± .01	0.53 ± .01	0.160 ± .012
Potassium (sample %)					
our value	1.00 ± .04	1.47 ± .09	0.98 ± .04	2.07 ± .06	2.365 ± .046
reference value	1.10 ± .03	1.67 ± .06	1.10 ± .05	2.19 ± .05	2.463 ± .122
Phosphorus (sample %)					
our value	0.17 ± .006	0.16 ± .002	0.12 ± .005	0.21 ± .006	0.446 ± .007
reference value	0.17 ± .004	0.17 ± .006	0.13 ± .01	0.22 ± .006	0.452 ± .016
Sodium (sample ppm)					
our value	76.9 ±3.0	54.0 ±1.7	716 ±46	26.9 ±1.4	2975 ±33
reference value	232 ±41	299 ±108	761 ±72	362 ±137	2594 ±617
Manganese (sample ppm)					
our value	104 ±1.9	93.1 ±3.3	28.5 ±1.5	80.2 ±4.2	17.9 ± .54
reference value	106 ±5.7	104 ±11	33.0 ±2.3	80.0 ±5.0	14.9 ±1.8
Iron (sample ppm)					
our value	299 ±43	211 ±37	513 ±42	294 ±30	144 ±12
reference value	245 ±17	197 ±24	456 ±22	230 ±12	120 ±20
Copper (sample ppm)					
our value	11.3 ± .79	354 ±14	32.4 ±1.6	18.6 ± .7	6.30 ± .49
reference value	14.3 ±1.8	241 ±33	33.5 ±3.3	21.0 ±1.8	4.81 ± .74
Zinc (sample ppm)					
our value	21.6 ± .9	28.3 ±1.3	76.7 ±4.0	31.2 ± .7	35.6 ±1.2
reference value	25.8 ±3.1	41.4 ±10	76.9 ±6.4	32.0 ±2.9	31.9 ±4.8
Number of analyses or laboratories					
our value	6–8	5–7	4–6	6–8	5–7
reference value	5–18	5–18	5–18	5–18	38–88

*fruit tree samples were redried after analyses were completed and lost weight accordingly: apple 6.6%, cherry 7.1%, citrus 5.8%, and peach 5.7%.

1. reference samples and values provided by Kenworthy, *et al.* (1956).
2. reference sample and values provided by Bowen (1967).

results. Too often this is not done. To test our methods of analysis for plant tissues, we obtained fruit tree leaf tissue from Dr. A. L. Kenworthy, Michigan State University and kale *(Brassica oleracea)* leaf tissue from Dr. H. J. M. Bowen, The University at Reading. Both of these reference standards have been chemically analyzed by several independent laboratories and the results have been published (Kenworthy and Miller 1956; Bowen 1967). A comparison of our results with the published values for these samples is given in Table 2.

Most of our values for the Michigan State fruit tree samples except for iron, are lower than the reference values; however, the samples were not redried prior to our analyses. When we dried these samples they lost 5.7 to 7.1% in weight. Adjustment of our results accordingly produces good agreement with the reference results, except for iron and sodium. The standard error for our iron analyses is large, but it would appear that our results are slightly higher in comparison with the reference samples, whereas for sodium our standard error indicates that our analyses are consistent and contamination is negligible since our values are lower than the reference values. Another point that must be considered is that the reference values were obtained at least 10 years before our analyses and it is conceivable that the plant tissue deteriorated or was not completely homogenous throughout all this time.

Since the kale sample became available more recently, it provided another check on our analytical procedures. Our values for calcium, magnesium, potassium and phosphorus agreed very well with the published values (Table 2). Our values for the other ions are somewhat higher than the published values, but the error limits for our analyses are very much smaller than for the published values (e.g. see sodium). Also, since our values for these ions were lower than the published values for the fruit tree samples, except for iron, our procedures appear to be reasonably accurate as well as relatively very consistent internally and free from spurious contamination.

Results and Discussion

VARIOUS plant tissue analyses for 6 tree species are presented in Table 3, and analyses of some 29 herbaceous terrestrial plants are given in Tables 4 and 5.

Tree Vegetation

The most abundant elements, in terms of relative concentration, in trees of the Hubbard Brook Experimental Forest are N, Ca, and K (Table 3). The highest concentrations of chemical elements are found in the current growth (leaves and current twigs). Bark also has relatively high concentrations, particularly for Ca, Na, Mn and Zn. In contrast the woody tissues have low concentrations of nutrient elements. Considering the deciduous nature of the major species in this forest, the unequal distribution of elements in these plant tissues is of great importance in nutrient cycling within the ecosystem. However, total assessment of the internal nutrient cycling must be based upon relative biomass of the various tissues. Also leaf tissue may lose appreciable amounts of certain elements by translocation and leaching before the abscission layers are formed (see Gosz *et al, 1970).*

Branch samples contained much lower concentrations of the various elements than current twigs (Table 3). Both current twig and branch samples consisted of wood and bark. Since the chemical content of bark is much greater than wood, the branch sample concentration may have been "diluted" by a relatively greater percentage of wood in the sample. Young and Guinn (1966) also found, for similar species in Maine, that the percent of each element in bark greatly exceeds the percent in wood for the same portion of a tree. They also infer that on-site (forest) bark removal from forest products, rather than at the processing plant, may have a relatively great effect in conserving nutrients within the forest ecosystem.

Yellow birch has relatively much higher concentrations of zinc in all tissues, particularly leaves, current twigs and bark, than any other tree species at Hubbard Brook (Table 3). Young *et al,* (1965) reports that white birch had 3-10 times as much zinc, for entire trees of the same size, as hemlock, red spruce, balsam fir, white pine, red maple and aspen in Maine. Gerloff *et al,* (1966) show a selective capacity for zinc absorption and accumulation in several species of Betula in Wisconsin, and Stone (1968) refers to these species as "zinc accumulator species." Except for leaf tissue, yellow birch at Hubbard Brook also has less ash weight than any other hardwood species (Table 3).

Even though foliage was not washed prior to analysis, the iron concentrations (Table 3) are only slightly higher than the minimal values cited by Stone (1968, p. 150) for closely related species. This would seem to be further evidence that contamination from road dust is negligible in the Hubbard Brook Ecosystem. However, roots of all species had relatively high concentrations of iron (Table 3). This may represent contamination from soil particles, although great care was taken to avoid this. The roots from hardwood species seemed to contain appreciably more iron than the coniferous species.

There was relatively little variation with elevation in chemical concentration for tissues of tree species at Hubbard Brook. This is indicated by the small standard errors in most cases. Some variations were observed; for example, leaf tissue from the major hardwood species contained 5 to 10-fold lower concentrations of manganese at lower elevations than at higher elevations. There also was less manganese in most of the other tissues for these species at lower elevations.

Safford and Young (1968) have reported significant differences in the nutrient content of red spruce foliage in trees growing on different soil series as well as from different sampling sites within a soil series. Within the Hermon Series, which is the soil series at Hubbard Brook, they found that aluminum was the only element that varied significantly from place to place. We didn't analyze for aluminum in plant tissue at Hubbard Brook, but our values for phosphorus and potassium in red spruce foliage were 1.6 to 2.0-fold less and manganese was more than 2-fold greater than those reported by Safford and Young (1968) for red spruce growing on soils of the Hermon Series in Maine. These comparative data apparently support the view that the nutrient aspects of a site may be estimated from the chemical analysis of foliage (e.g. Mitchell 1935; 1936).

The branches, and possibly current twigs of red spruce contained more iron than the branches of other species, although iron was not significantly more abundant in any other red spruce tissue, relative to the other species (Table 3). Similarly, Young and Guinn (1966) found that "tiny branches" from red spruce in Maine contained relatively very high concentrations of iron.

Variations in the chemical content of foliage from tree species during the growing season is well known (e.g. Mitchell 1936, Guha and Mitchell 1966). Chemical analyses of leaf samples from sugar maple, beech and yellow birch trees collected in July, August and September 1965 from the lower portion of the experimental watersheds provide some additional information in this regard (Fig. 1). "Sun" leaves, exposed near the top of the crown, and "shade" leaves from lower down or from the interior of the crown were analyzed. There

TABLE 3. CHEMICAL COMPOSITION OF SOME TREE SPECIES FROM THE HUBBARD BROOK EXPERIMENTAL FOREST. VALUES GIVEN ARE MEANS AND STANDARD ERRORS OF THE MEAN BASED UPON DRY WEIGHT ANALYSES.
SAMPLES WERE COLLECTED IN AN AREA ADJACENT TO WATERSHED 6 DURING JULY AND AUGUST 1966.

	Sample %						Sample ppm						
Species	Ca	Mg	K	P	S	N	Na	Fe	Zn	Cu	Mn	% Ash Wt.	No. of Sample
						Whole Leaves							
Acer saccharum	0.60 ± .11	0.12 ± .01	1.01 ± .05	0.18 ± .02	0.206 ±.014	2.19 ±.08	16.3 ± 1.75	119 ± 12.8	51.7 ± 4.8	9.01 ± .75	1740 ±579	5.65 ± .38	6
Betula alleghaniensis	0.88 ± .08	0.25 ± .03	1.14 ± .12	0.20 ± .00	0.143 ±.005	2.78 ±.09	19.7 ± 2.05	120 ± 7.94	334 ±29.3	9.69 ± .25	1920 ±350	5.96 ± .54	6
Fagus grandifolia	0.55 ± .04	0.15 ± .01	0.88 ± .06	0.18 ± .02	0.166 ±.014	2.23 ±.09	15.4 ± 2.00	118 ± 11.1	25.5 ± 1.70	10.6 ± .35	1200 ±254	4.68 ± .50	6
Acer spicatum	0.75 ± .06	0.13 ± .01	1.33 ± .12	0.20 ± .00	0.195 ±.004	2.49 ±.12	22.0 ± 5.24	133 ± 10.5	45.4 ± 5.60	9.7 ± .81	1600 ±174	5.35 ± .37	5
Picea rubens	0.30 ± .03	0.06 ± .002	0.49 ± .05	0.10 ± .00	0.110 ±.004	1.26 ±.05	11.3 ± 1.47	67.3 ± 3.09	20.7 ± 1.17	5.77 ± .48	2070 ±425	2.83 ± .06	5
Prunus pensylvanica	1.02 ± .21	0.29 ± .02	1.71 ± .12	0.24 ± .02	—	2.73	10.1 ± 3.01	80.6 ± 12.2	24.4 ± 2.0	8.64 ± .90	150.8 ±26.8	4.11 ±1.13	5#
							Current Twigs (bark and wood)						
A. saccharum	1.62 ± .26	0.10 ± .004	0.93 ± .08	0.18 ± .05	—	—	39.8 ±10.0	53.8 ± 7.29	62.4 ± 2.54	12.8 ±1.37	1330 ±315	5.87 ± .62	6
B. alleghaniensis	0.96 ± .08	0.16 ± .01	0.52 ± .06	0.21 ± .05	—	—	28.4 ± 4.34	57.5 ± 7.00	226 ±22.0	13.5 ±1.59	1250 ±327	2.74 ±1.02	6
F. grandifolia	1.23 ± .15	0.23 ± .03	0.58 ± .04	0.20 ± .04	—	—	20.7 ± 5.30	42.2 ± 6.67	39.9 ± 3.03	15.0 ± .65	1390 ±296	5.39 ± .79	6
A. spicatum	1.17 ± .17	0.12 ± .01	0.56 ± .09	0.14 ± .02	—	—	24.3 ± 3.73	47.1 ± 4.99	62.9 ± 8.92	8.99 ± .91	1540 ±181	4.52 ± .52	5
P. rubens	0.09 ± .03	0.09 ± .01	0.81 ± .03	0.20 ± .00	—	—	16.2 ± 2.32	74.7 ± 6.96	40.3 ± 2.92	20.8 ±3.30	1240 ±171	2.44 ± .06	5
P. pensylvanica	0.49 ± .04	0.08 ± .007	0.63 ± .05	0.10 ± .004	—	0.65	6.0 ± 0.82	15.8 ± .6	21.9 ± 2.3	4.92 ± .36	36.7 ±4.01	3.36 ±1.02	5#
							Branch (bark and wood)						
A. saccharum	0.43 ± .06	0.03 ± .002	0.17 ± .01	0.07 ± .01	0.045 ±.002	0.37 ±.03	6.75 ± .64	24.0 ± 2.87	18.9 ± 1.05	4.25 ± .65	582 ±129	1.66 ± .17	6
B. alleghaniensis	0.42 ± .04	0.03 ± .003	0.10 ± .01	0.03 ± .002	0.036 ±.004	0.44 ±.02	11.1 ± 1.31	22.9 ± 2.34	154 ±14.2	4.14 ± .30	367 ±60.9	1.52 ± .09	11
F. grandifolia	0.47 ± .05	0.03 ± .002	0.12 ± .01	0.03 ± .005	0.046 ±.008	0.30 ±.03	6.79 ± .84	19.4 ± 1.20	21.1 ± 3.28	4.81 ± .28	269 ±45.2	1.88 ± .19	6
A. spicatum	0.46 ± .04	0.05 ± .002	0.18 ± .01	0.10 ± .00	0.030 ± ∞	0.48 ± ∞	7.45 ± .79	39.6 ± 7.67	44.9 ± 4.52	5.12 ± .70	662 ±51.1	1.85 ± .13	5
P. Rubens	0.25 ± .03	0.05 ± .003	0.34 ± .06	0.10 ± .00	—	—	14.6 ± 1.22	172 ± 18.2	58.9 ± 2.42	7.80 ± .66	148 ±17.9	1.91 ± .10	5
P. pensylvanica	0.41 ± .04	0.04 ± .000	0.17 ± .02	0.03 ± .003	—	0.30	5.56 ± 0.61	15.5 ± 1.5	27.4 ± 2.9	3.74 ± .46	73.3 ±10.0	1.19 ± .16	5#

Species	Sample % Ca	Mg	K	P	S	N (Whole leaves)	Sample ppm Na	Fe	Zn	Cu	Mn	% Ash Wt.	No. of Samples
Bark (trunk)													
A. saccharum	1.41 ± .20	0.06 ± .005	0.29 ± .03	0.03 ± .002	0.064 ±.005	0.55 ±.02	93.3 ±20.9	55.4 ± 8.64	29.4 ± 2.54	6.13 ± .52	940 ±217	4.54 ± .62	7
B. alleghaniensis	0.96 ± .14	0.04 ± .004	0.13 ± .003	0.03 ± .002	0.042 ±.006	0.60 ±.05	48.6 ± 4.62	17.7 ± 1.81	259 ±22.7	4.01 ± .30	655 ±149	2.63 ± .18	7
F. grandifolia	2.53 ± .23	0.05 ± .007	0.22 ± .03	0.04 ± .004	0.074 ±.012	0.75 ±.04	68.1 ±18.1	42.1 ± 4.20	9.57 ± 1.30	13.6 ±2.0	954 ±230	7.83 ± .63	6
A. spicatum	1.36 ± .08	0.06 ± .004	0.16 ± .01	0.09 ± .012	0.078 ±.008	0.76 ±.05	132 ±17.4	79.9 ± 24.7	97.2 ±16.2	7.07 ± .84	1370 ±232	4.01 ± .21	5
P. rubens	0.68 ± .05	0.04 ± .006	0.18 ± .02	0.04 ± .014	0.053 ±.007	0.35 ±.02	106 ±35.8	65.1 ± 13.9	103 ±10.1	4.60 ± .61	1670 ±202	2.59 ± .18	5
P. pensylvanica	1.11 ± .12	0.11 ± .008	0.48 ± .03	0.10 ± .004	—	0.76	10.3 ± .66	22.2 ± 2.0	29.9 ± 3.2	5.67 ± .56	114 ± 9.0	3.30 ± .19	5#
Light Wood (trunk)*													
A. saccharum	0.10 ± .02	0.02 ± .002	0.07 ± .01	0.01 ± .001	0.011 ±.001	0.098 ±.009	7.46 ± 2.55	21.4 ± 10.2	7.73 ± .86	1.21 ± .32	138 ±34.1	0.42 ± .07	6
B. alleghaniensis	0.06 ± .01	0.01 ± .002	0.03 ± .004	0.01 ± .001	0.018 ±.002	0.092 ±.010	5.64 ± 1.32	31.5 ± 10.6	32.2 ± 2.62	1.69 ± .12	131 ±33.0	0.23 ± .02	6
F. grandifolia	0.06 ± .004	0.02 ± .002	0.07 ± .007	0.01 ± .001	0.026 ±.004	0.110 ±.008	7.31 ± 1.12	12.2 ± 3.63	7.06 ± 2.73	2.33 ± .11	82.1 ±10.8	0.38 ± .02	10
A. spicatum	0.09 ± .03	0.01 ± .002	0.04 ± .005	0.008 ± .002	0.015 ±.003	0.069 ±.007	7.15 ± .41	5.85 ± 1.26	11.7 ± 2.21	1.20 ± .17	142 ±24.3	0.29 ± .01	5
P. rubens	0.06 ± .01	0.01 ± .000	0.02 ± .00	0.001 ± .0003	0.015 ±.001	0.055 ±.008	1.63 ± .26	5.94 ± 1.54	12.5 ± 1.78	1.40 ± .25	298 ±44.2	0.27 ± .03	4
P. pensylvanica	0.31 ± .09	0.03 ± .005	0.26 ± .06	0.04 ± .006	—	0.24	6.67 ± 1.82	10.3 ± 1.0	8.53 ± 1.46	4.37 ± .55	12.9 ± 1.2	0.99 ± .11	5#
Dark Wood (trunk)*													
A. saccharum	0.32 ±0.1	0.06 ± .02	0.30 ± .04	.004 ± .001	0.009 ±.001	0.097 ±.013	9.75 ± 3.25	17.8 ± 11.4	9.69 ± 1.94	0.50 ± .00	468 ±65.6	1.39 ± .17	2
B. alleghaniensis	0.09 ± .01	0.02 ± .003	0.11 ± .08	0.01 ± .003	0.020 ±.003	0.097 ±.008	9.50 ± 4.00	15.5 ± 3.72	40.6 ± 4.85	1.75 ± .32	200 ±45.9	0.64 ± .15	3
F. grandifolia	0.08 ± .01	0.02 ± .002	0.10 ± .01	0.002 ± .0001	0.028 ±.004	0.12 ±.008	5.72 ± 1.49	10.6 ± 2.10	7.69 ± 2.27	1.52 ± .24	73.8 ±12.0	0.44 ± .03	9
A. spicatum	0.08 ± .02	0.02 ± .00	0.07 ± .00	0.004 ± .000	0.014 ± ∞	0.12 ± ∞	12.2 ± 1.51	6.87 ± .62	13.1 ± .61	1.38 ± .13	158 ± 6.22	0.45 ± .005	2
Roots (bark and wood)													
A. saccharum	0.25 ± .07	0.05 ± .01	0.27 ± .08	0.37 ± .09	0.073 ±.016	0.71 ±.09	125 ±19.1	1475 ±345	46.1 ± 5.8	6.06 ± .40	382 ±68.2	3.28 ± .22	6
B. alleghaniensis	0.37 ± .04	0.04 ± .003	0.14 ± .01	0.08 ± .01	0.066 ±.019	0.61 ±.06	122 ±14.2	805.8 ±149.5	117.8 ± 9.6	7.64 ± .51	277 ±60.3	2.48 ± .11	5
F. grandifolia	0.36 ± .07	0.05 ± .002	0.24 ± .02	0.12 ± .02	0.044 ±.011	0.64 ±.09	145 ±63.6	1062 ±147	46.9 ± 5.4	7.48 ± .63	272 ±72.5	3.56 ± .46	5
A. spicatum	1.25 ± .70	0.07 ± .01	0.23 ± .01	0.10 ± .00	0.062 ±.013	0.82 ±.16	198 ±40.8	351.2 ±148.4	74.6 ±13.1	5.77 ± .18	556 ±150	2.67 ± .22	5
P. rubens	0.59 ± .06	0.05 ± .003	0.26 ± .03	0.04 ± .002	0.046 ±.013	0.31 ±.03	104 ±10.9	184.8 ± 20.5	118.9 ±15.2	7.53 ± .17	802 ±66.6	2.33 ± .21	5
P. pensylvanica	0.46 ± .08	0.05 ± .003	0.34 ± .04	0.05 ± .004	—	0.35	449 ±113	74.1 ± 22.8	28.1 ± 2.8	5.34 ± .49	43.9 ±19.6	1.57 ± .15	5#

*Light wood is equivalent to sapwood, while dark wood is either heart wood or wood discolored by disease organism.
#Samples from lower third of the watershed only.

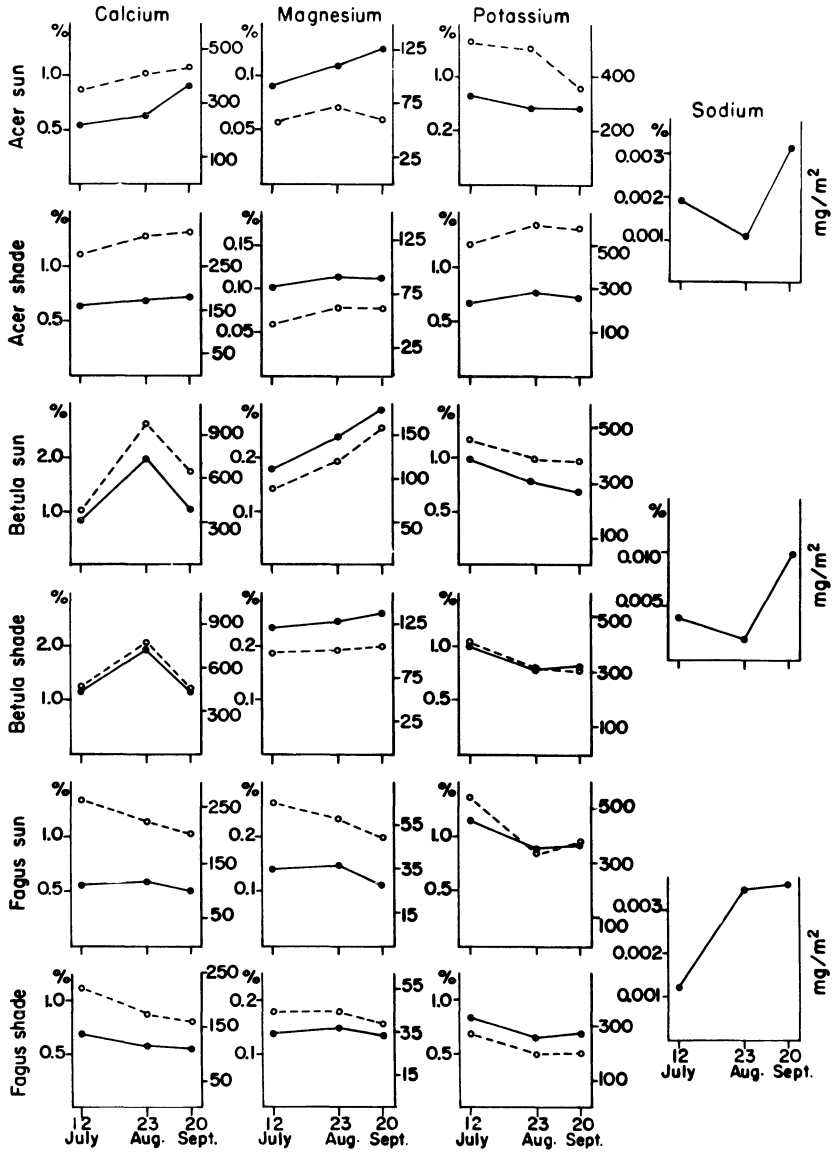

FIGURE LEGEND

FIGURE. 1. Seasonal changes in chemical content for sun and shade leaves of three species collected in the Hubbard Brook Experimental Forest during 1965. Leaves collected on 23 August and 20 September were from the same tree. Sodium analyses were not differentiated as to sun and shade leaves .—. is sample % on a dry weight basis, o—o is mg/m^2 of leaf area. The left hand scale is % dry weight, while the right hand scale is mg/m^2 (modified from Gosz, *et al.* 1970).

were appreciable changes in concentration of calcium, magnesium, potassium and sodium in these leaves during the summer, and presumably there would have been even greater differences if leaves earlier and later in the season had been analyzed (e.g. Hoyle 1965). Sodium concentration was particularly variable in all species (Fig. 1). Calcium concentration was somewhat more variable in yellow birch than in the other two species. An August maximum in calcium content for leaves of yellow birch also was shown by Hoyle (1965) for trees of 7 inches (17.8 cm) dbh in New Hampshire. It should be noted that the potassium content in beech shade leaves seems to be consistently lower than in sun leaves, whereas the concentration of calcium, magnesium and potassium were similar in other sun and shade leaves from the same species.

It is of interest to compare the concentrations in 1965 with those in 1966 (Fig. 1 and Table 3). The agreement is surprisingly good. However, there were small differences, e.g. potassium content was lower in 1965 in sugar maple and yellow birch, calcium was higher in 1965 in yellow birch and sodium was somewhat higher in 1965 in yellow birch and beech. Hoyle's (1965) calcium and magnesium values for yellow birch leaves collected on Hermon soil in the Bartlett Experimental Forest (White Mountains, New Hampshire) agree very closely with our values.

Due to seasonal changes in chemical content of foliage from deciduous trees, it is generally agreed that samples of leaf tissue for chemical analysis should be obtained late in the growing season (see review by Kramer and Kozlowski 1960). However, Leaf (1968) suggests that although the relative stability of nutrient levels is greatest in the autumn foliage, such nutrient levels may not characterize the physiologically most active period. Such considerations are very important in calculations of nutrient budgets for a forest where tissue concentrations are multiplied by biomass values. For example, the absolute amount of an element might be the same even though the concentration was decreasing, if the biomass were increasing. The absolute amount might increase if 1) the concentration were increasing and the biomass were increasing or 2) if the concentration remained constant while the biomass was increasing, and so on.

Herbaceous Vegetation

The data given in Table 4 represent averages for plants, collected throughout the watershed-ecosystem. Considering the size of the standard error for most of the analyses, these probably represent good average values for the ecosystem. However the concentration of some nutrients differed markedly in the elevational subdivisions (Siccama, *et al.* 1970). The concentrations of phosphorus,

TABLE 4. CHEMICAL COMPOSITION FOR SHOOTS OF SOME HERBACEOUS SPECIES FROM THE HUBBARD BROOK EXPERIMENTAL FOREST. VALUES GIVEN ARE MEANS AND STANDARD ERRORS OF THE MEAN BASED UPON DRY WEIGHT ANALYSES OF COMPOSITE SAMPLES COLLECTED 14–25 JULY 1967 FROM EACH OF THE UPPER, MIDDLE AND LOWER THIRDS OF WATERSHED 6.

	Sample %						Sample ppm					% Ash	#Samples
Species	Ca	Mg	K	P	S	N	Na	Fe	Zn	Cu	Mn		
1. Athyrium felix-femina	0.81 ±.06	0.38 ±.04	3.04 ± .10	0.18 ±.03	0.19 ±.008	2.53 ±.15	19.9 ± 1.41	179 ±73.5	44.3 ± 5.42	13.6 ± 1.70	221 ±69.0	114 ± .91	3
2. Dennstaedtia punctilobula	0.21 ±.06	0.24 ±.04	2.03 ± .62	0.18 ±.02	0.21 ±.02	2.82 ±.46	10.8 ± 1.53	114 ±20.6	62.9 ±13.0	8.85 ± .97	1341 ±317	7.3 ±1.30	3
3. Dryopteris noveboracensis	0.54 ±.22	0.42 ±.02	2.78 ± .30	0.17 ±.03	0.31[a]	2.38[a]	15.7 ± 3.70	117 ± 8.95	41.4 ± 1.35	12.5 ± 2.00	1090 ±155	8.6 ± .02	2[d]
4. Dryopteris phegopteris	0.61 ±.06	0.43 ±.04	2.81 ± .33	0.15 ±.02	0.33 ±.01[a]	2.25 ±.08[a]	57.1 ±12.0	163 ±22.5	50.5 ± 3.90	11.5 ± 1.69	357 ±42.1	11.2 ± .87	3
5. Dryopteris spinulosa	0.37 ±.01	0.43 ±.06	2.58 ± .13	0.20 ±.003	0.22 ±.008	2.47 ±.11	15.8 ± .59	81.4 ±13.4	104 ±16.0	14.2 ± .92	1366 ±106	7.13 ± .25	3
6. Lycopodium lucidulum	0.06 ±.005	0.12 ±.003	1.41 ± .04	0.10 ±.009	0.17 ±.009	2.01 ±.01	22.2 ± 2.48	218 ±77.6	35.6 ± 4.51	7.58 ± 1.02	296 ±187	3.97 ± .27	3
7. Osmunda claytoniana	0.29 ±.006	0.13 ±.008	2.88 ± .04	0.18 ±.007	0.21[a]	2.41[a]	12.1 ± 1.14	54.2 ± 6.80	64.2 ± 1.78	7.89 ± .29	1049 ±66.9	7.20 ± .15	1[a,a]
8. Aralia nudicaulis	0.80 ±.11	0.28 ±.02	1.71 ± .21	0.22 ±.02	0.19 ±.006	2.32 ±.16	12.0 ± .74	105 ± 8.72	74.3 ± 6.79	9.12 ± .87	2668 ±1080	6.26 ± .39	3
9. Arisaema atrorubens	0.92	0.24	1.78	0.21	0.22	—	25.5	242	199	7.1	255	7.78	1[b]
10. Aster acuminatus	0.67 ±.04	0.30 ±.04	2.92 ± .14	0.19 ±.02	0.18 ±.01	2.14 ±.06	21.6 ± 5.90	278 ±70.2	214 ± 7.39	15.7 ± .49	2414 ±453	9.43 ± .49	3
11. Carex intumescens	0.18 ±.003	0.14 ±.01	1.90 ± .34	0.22 ±.04	0.29 ±.002	2.84 ±.03	12.6 ± 2.30	122 ±13.3	77.4 ±10.4	18.8 ± 3.90	1037 ±59.5	8.05 ± .16	2[d]
12. Carex leptoneruia	0.27 ±.12	0.15 ±.02	3.23 ± .51	0.15 ±.01	0.22 ±.004	2.86 ±.09	14.3 ± .50	272 ±123	93.3 ± 9.25	27.4 ±14.6	666 ±145	11.5 ± .73	2[e]
13. Clintonia borealis	1.10 ±.53	0.35 ±.05	4.47 ± .35	0.16 ±.02	0.14 ±.01	2.29[f]	25.6 ± 4.74	271 ±146	47.7 ± 7.74	20.4 ±11.3	1160 ±240	12.9 ±1.18	
14. Cornus canadensis	3.09	0.45	1.35	0.25	0.22	1.79	8.55	101	45.8	5.03	529	10.3	1[a]

Species	Sample % Ca	Mg	K	P	S	N	Sample ppm Na	Fe	Zn	Cu	Mn	% Ash	# Samples
15. Galium triflorum	1.70 ±.94	0.27 ±.13	2.14 ± .94	0.20 ±.005	0.31[f]	2.83[f]	20.3 ± 2.05	109 ±14.7	294 ±137	12.0 ± 1.80	318 ±77.0	13.8[f]	2[e]
16. Maianthemum canadense virginiana	0.86 ±.30	0.30 ±.04	4.99 ±1.82	0.22 ±.005	0.17 ±.003	2.40 ±.07	10.9 ± 1.70	136 ± 7.30	87.5 ± 3.20	7.62 ± .56	1444 ±322	9.97 ± .76	2[d]
17. Medeola	0.46 ±.05	0.34 ±.06	2.24 ± .02	0.13 ±.005	0.22[b]	2.30[b]	18.0 ± 3.15	186 ±18.0	88.2 ± 8.15	16.4 ± 7.83	234 ±17.2	7.12 ± .03	2[e]
18. Mitchella repens	1.14 ±.43	0.35 ±.10	1.15 ± .37	0.11 ±.005	0.15[b]	—	14.7 ± 3.20	128 ±12.7	59.8 ± 2.10	7.94 ± .25	656 ±275	13.5 ±5.70	2[e]
19. Oxalis montana	0.60 ±.13	0.40 ±.11	2.92 ± .56	0.25 ±.02	0.33 ±.001[d]	—	28.8 ± 6.19	302 ±95.8	84.3 ± 7.16	9.56 ± .84	1330 ±214	8.52 ±1.00	3
20. Polygonatum pubescens	0.52	0.14	5.45	0.32	—	—	16.6	117	63.7	16.6	1558	8.82	1[b]
21. Smilacina racemosa	0.99	0.20	2.73	0.13	0.17 ±.006[c]	2.27 ±.005[c]	8.9	136	43.6	7.9	620	9.19 ± .41[c]	1[b]
22. Solidago macrophylla	0.98	0.41	5.38	0.31	0.18	2.58	25.5	120	86.0	17.2	3488	9.64	1[a]
23. Streptopus roseus	0.81 ±.29	0.28 ±.03	3.39 ± .08	0.15 ±.02	0.15 ±.005	2.00 ±.04[c]	15.7 ± 2.63	164 ±26.1	233 ±45.5	9.48 ± .70	1069 ±336	11.1 ± .43	3
24. Trientalis borealis	1.16	0.44	3.03	0.17	0.13	2.02	13.1	129	36.4	7.76	485	5.32	1[a]
25. Trillium erectum	0.77 ±.08	0.22 ±.02	3.21 ± .27	0.15 ±.006	0.15 ±.009[e]	2.47 ±.15[e]	9.93 ± .67	119 ±10.5	75.2 ± 9.29	9.33 ± .37	764 ±276	10.2 ± .40	3
26. Trillium undulatum	1.31 ±.29	0.47 ±.14	7.41 ±2.92	0.16 ±.02	0.32[b]	—	13.1 ± .85	132 ± 6.79	21.8 ± 1.95	4.82 ± 1.39	156 ±39.1	14.1 ± .55	3
27. Uvularia sessilifolia	0.85 ±.04	0.24 ±.02	2.22 ± .24	0.20 ±.06	0.18 ±.01	2.54 ±.11	8.93 ± 1.41	114 ±12.0	31.2 ± 2.20	11.3 ± .40	434 ±128	6.31 ± .61	3
28. Viola incognita	0.78 ±.04	0.83 ±.005	3.68 ±1.30	0.22 ± 0	0.26[f]	3.22[f]	20.9 ± 4.05	142 ±12.8	302 ±54.2	8.19 ± .23	1316 ±764	11.6 ± .94	2[e]
29. Viola rotundifolia	0.64 ±.15	0.47 ±.05	4.69 ± .62	0.12 ±.005	0.23[f]	2.76[f]	39.5 ± 4.65	514 ±96.2	398 ±19.1	8.84 ± .91	526 ±147	16.4 ±1.95	2[e]

a—sample from upper third only
b—sample from lower third only
c—sample from lower and middle thirds only
d—sample from middle and upper thirds only
e—sample from upper and lower thirds only
f—sample from middle third only
*—5 replicates

TABLE 5. CHEMICAL COMPOSITION OF REPLICATE SAMPLES FOR SHOOTS OF HERBACEOUS SPECIES COLLECTED FROM THE LOWER THIRD OF WATERSHED 6 ON 7 SEPTEMBER 1967. VALUES ARE MEANS AND STANDARD ERRORS OF THE MEAN BASED ON DRY WEIGHT ANALYSES.

	Sample %						Sample ppm						#
Species	Ca	Mg	K	P	S	N	Na	Fe	Zn	Cu	Mn	% Ash	Sample
Aster acuminatus	0.70 ±.05	0.24 ±.02	2.30 ±.23	0.17 ±.01	0.18 ±.01	2.12 ±.06	19.1 ±2.51	261 ±43.8	251 ±13.4	19.3 ±1.18	2703 ±142	9.07 ±.24	4–5
Dryopteris spinulosa	0.34 ±.04	0.36 ±.04	2.41 ±.27	0.20 ±.005	0.22 ±.008	2.28 ±.04	18.1 ± .73	107 ± 3.73	106 ± 5.98	14.4 ± .68	1831 ±74.2	6.94 ±.14	5
Smilacina racemosa	0.84 ±.07	0.16 ±.01	2.99 ±.25	0.13 ±0.0	0.15 ±.01	1.88 ±.06	11.0 ± .41	117 ± 9.30	42.1 ± 2.90	11.7 ±1.25	968 ±79.4	9.71 ±.24	4–5
Lycopodium lucidulum	0.06 ±.005	0.16 ±.007	1.71 ±.14	0.09 ±.004	0.18 ±.005	2.06 ±.07	24.3 ±1.15	168 ± 8.42	38.0 ± 1.84	8.14 ± .70	93.7 ± 9.48	4.60 ±.09	5
Dennstaedtia punctilobula	0.33 ±.03	0.33 ±.02	3.64 ±.27	0.13 ±.003	0.20 ±.016	2.64 ±.10	20.7 ±2.87	117 ±21.2	82.0 ± 4.32	20.1 ±9.21	1969 ±126	8.97 ±.19	5
Clintonia borealis	1.06 ±.03	0.35 ±.01	4.61 ±.23	0.15 ±.01	0.13 ±.004	2.01 ±.02	24.7 ±1.90	134 ± 8.80	39.2 ± 1.75	10.9 ± .79	1371 ±45.9	12.9 ±.34	4–5
Lycopodium lucidulum*	0.07 ±.007	0.17 ±.006	1.71 ±.05	0.10 ±.002	—	—	25.2 ±1.47	175 ±18.9	42.2 ± 1.30	9.38 ± .22	125 ± 5.80	4.69 ±.04	5*

*A replicate analysis of one sample from the above

TABLE 6. CHEMICAL COMPOSITION OF SOME SPRING HERBS FROM THE HUBBARD BROOK EXPERIMENTAL FOREST. SAMPLES WERE COLLECTED DURING LATE MAY, 1967 FROM THE AREA OF WATERSHED 6. VALUES GIVEN ARE MEANS AND STANDARD ERRORS OF THE MEAN BASED UPON DRY WEIGHT ANALYSES. VALUES IN ( ) INDICATE THE NUMBER OF SAMPLES ANALYZED.

	Sample %				Sample ppm					% Ash
Species	Ca	Mg	K	P	Na	Fe	Zn	Cu	Mn	
Trillium erectum										
shoot (3)	0.57 ±.17	0.21 ±.02	3.28 ± .13	0.22 ±.02	81.9 ±28.9	193 ±99.1	92.3 ±19.1	12.8 ±1.20	302.8 ± 88.1	10.5 ± .27
rhizome (3)	0.65 ±.21	0.11 ±.01	1.00 ± .14	0.09 ±.01	361 ±81.6	358 ±226	252 ±113	9.10 ± .54	603.0 ±397	8.61 ± 2.1
T. undulatum										
shoot (3)	0.38 ±.05	0.17 ±.02	3.69 ± .22	0.38 ±.04	37.4 ± 9.20	180 ±51.2	40.5 ± 2.64	10.4 ±3.03	76.35 ± 1.45	14.0 ± .47
rhizome (3)	0.48 ±.05	0.11 ±.02	1.01 ± .14	0.15 ±.03	220 ±75.6	1350 ±236	157 ±68.9	9.49 ±1.77	138.5 ± 41.8	22.3 ±12.6
Erythronium americanum										
shoot (3)	0.20 ±.02	0.14 ±.01	1.94 ± .06	0.17 ±.02	130 ±25.7	254 ±114	63.2 ± 5.19	11.4 ±1.57	98.62 ± 11.5	7.36 ± .75
bulb & roots (1)	0.25	0.04	0.46	0.04	117	526	43.3	3.87	13.93	10.8
root (2)	0.06 ±.02	0.10 ±.04	0.44 ± .04	0.05 ±.01	143 ±67.3	1040 ±857	37.6 ±13.5	7.01 ±1.76	49.1 ± 16.6	6.95 ± 5.1
Uvularia sessilifolia										
shoot (3)	0.31 ±.01	0.15 ±.01	3.07 ± .34	0.34 ±.04	74.2 ± 6.41	262 ±68.0	94.9 ±14.1	28.0 ±3.93	127.1 ± 7.98	7.51 ± .82
rhizome (3)	0.56 ±.06	0.16 ±.01	1.10 ± .12	0.10 ±.01	257 ±38.0	301 ±25.6	95.8 ±28.6	19.8 ±4.90	333.4 ±127	4.90 ± .25
Viola incognita										
shoot (1)	0.31	0.30	4.34	0.30	75.8	306	196	19.0	521.2	15.9
root (1)	3.37	0.37	1.08	0.16	457	1030	345	23.2	325.3	12.0
Claytonia caroliniana										
shoot (2)	0.19 ±.02	0.54 ±.16	3.42 ±1.60	0.22 ±.04	119 ±37.0	366 ±210	243 ±42.6	11.1 ±2.34	234.9 ±116.5	10.9 ± 5.55
tuber (2)	0.05 ±.02	0.16 ±.04	0.52 ± .14	0.13 ±.03	70.2 ± 4.45	321 ±168	62.8 ± 4.05	5.30 ±2.40	95.40 ± 46.6	2.86 ± .90

calcium, magnesium and manganese were higher at upper elevations, whereas concentrations of zinc and iron were lower. These differences may reflect differences in soil chemistry in the watershed (Gagnon *et al, 1958).*

The herbaceous shoots contain relatively large amounts of potassium. In general the potassium concentration is 2 to 4 times higher than the concentration in leaves of tree species at Hubbard Brook and probably represents some measure of the capacity for selective uptake and accumulation by these herbaceous species. This is an important consideration in terms of internal nutrient cycling in these watershed-ecosystems. The concentration of zinc is variable, but is also somewhat higher in the herbaceous plants, particularly in the violets (V. *incognita* and V. *rotundifolia,* Table 4). Manganese concentrations were very variable between herbaceous species; the average values varied as much as 23-fold.

Replicate samples of 6 herbaceous species from the lower elevation of W6 were collected on 7 September to provide data on combined sampling and analytical errors. Also 5 replicate subsamples from one of these species were analyzed to indicate the magnitude of the analytical error (Table 5). These data, obtained for herbs in September, were useful also for comparing seasonal differences in nutrient concentrations. Relatively small differences were observed between these July and September dates (Tables 4 and 5). Considering chemical determinations for 6 species, the means (average watershed-ecosystem) for the July collections were within the 95% fiducial range of the September means (lower sub-division only) 44 times, while 15 of the July means were below the September fiducial range and 6 exceeded the range (Siccama *et al,* 1970). Thus, in contrast to the tree vegetation (Fig. ı and Guha and Mitchell 1966), there appears to be relatively little change in chemical concentration in herbs during the summer, although there may be considerable change in biomass.

It would appear from these data (Table 5) that the analytical errors contribute the major portion of the variability in analyses of iron, sodium, calcium and magnesium in herbs. However the combined sampling and analytical error produced a standard error $< 10\%$ of the mean in 86% of the cases. This would appear to be satisfactory for most computations related to nutrient budgets for ecosystems.

Spring Herbs

Plant parts from 6 of the early spring species were analyzed (Table 6). Four of these species also were collected and analyzed during the summer. A com-

parison of the results for the spring collection with values obtained for plants of the same species collected in the summer (Table 4) indicated some large differences in chemical content. Note that the chemical concentrations given for the spring collection (Table 6) represent the averages of analyses from individual plants, whereas the summer concentrations (Tables 4 and 5) are averages of samples composited from several plants. The plants collected in the spring had consistently higher concentrations of P, Na and Cu and consistently lower concentrations of Ca, Mg and Mn. Concentrations of the other elements were similar or variable. For example, concentrations of Zn were greater during spring in two species and lower in two others. The potassium concentration was lower in one species and the Fe concentration was higher in two species during the spring. Unfortunately analyses of N and S were not done on the spring collections. The ash weight percentages were very similar in spring and summer plants. Also of interest are comparisons between concentrations for above and below ground tissues (Table 6). For example there is consistently more K and P and generally more Mg in shoots than in roots. Except for *Claytonia caroliniana,* there are consistently higher concentrations of Na and Fe in below ground tissues. These differences are probably the result of selective accumulation, absorption and utilization as related to the different species and physiological activity during the relatively short and critical growing season for these plants (e.g. Gerloff *et al.* 1966).

Other Plants

Two aquatic plants from the Hubbard Brook Ecosystem were obtained (grab samples) and analyzed (Table 7). The alga, *Ulothrix zonata* (Weber and Mohr) Kutz, was growing in relatively very nutrient rich stream water in Watershed 2 (Likens, *et el.* 1970). The relatively high ash content seems to be typical of some species of chlorophyta (e.g. Birge and Juday 1922, Schuette and Hoffman 1922). Nevertheless the cation and phosphorus content of this alga do not appear to be relatively high in relation to other algae in lakes (e.g. Birge and Juday 1922). It appears that the upstream algae had a lower cation content; however, this may be due in part to increased insolubility during dry ashing. These samples had relatively very high insoluble ash content.

The aquatic moss, *Philonotis fontana* (Hedw.) Brid., which was growing on a rock in the stream of an undisturbed forest, was conspicuously low in Mg and K concentration (Table 7).

TABLE 7. CHEMICAL COMPOSITION OF A MOSS AND A GREEN ALGA FROM STREAMS TRIBUTARY TO HUBBARD BROOK.* VALUES GIVEN ARE MEANS AND ARE BASED UPON DRY WEIGHT ANALYSES.

	Sample %					Sample ppm					% Ash
Sample	Ca	Mg	K	N	P	Na	Fe	Zn	Cu	Mn	
Ulothrix zonata											
upper elevation	0.086	0.093	0.17	—	0.12	91.0	483	58.6	13.4	345	44.8
lower elevation	0.082	0.12	0.29	—	0.22	101	433	63.3	16.6	433	27.3
Philonotis fontana											
(aquatic moss)	0.16	0.03	0.04	0.95	0.10	64.6	—	—	—	—	31.4

*The moss was obtained during spring 1969 from an undistributed watershed and the alga was obtained during summer 1968 from a deforested watershed.

Summary and Conclusions

1. Chemical analyses for Ca, Mg, K, Na, Fe, Cu, Zn, Mn, N, S, P and ash content were made on various tissues of 6 tree species, 31 herbaceous species, 1 algal species and 1 species of aquatic moss from the Hubbard Brook Ecosystem. The samples for cation and phosphorus analysis were dry ashed at 500°C or less, and the cation concentrations were determined by atomic absorption spectrophotometry. Determination of P, N and S were made by standard procedures. Because of the rapidly increasing interest in studies of nutrient cycling, collection and analytical procedures are described in detail.

2. Some corrections were made to the tree tissue analyses to account for cations absorbed on an insoluble residue of silica, which resulted from the dry ashing procedure. The majority of the analytical results were unaffected; however, corrections were necessary for most root and bark samples, and for the copper and zinc results in general.

3. In general, our analytical results agreed very well with published results for reference plant tissue samples. Our results for iron were the most variable and disparate in this regard.

4. Nitrogen, calcium and potassium are the most abundant elements in trees at Hubbard Brook and they have their highest concentration in the current growth (leaves and current twigs). Yellow birch selectively accumulates zinc, particularly in the leaves, twigs and bark. Hardwood tree leaves contained 5 to 10-fold greater concentrations of manganese at higher elevations.

5. Appreciable changes in Ca, Mg, K and Na concentrations were observed in "sun" and "shade" leaves of sugar maple, beech and yellow birch during the summer months. However, there was good general agreement in cation content between leaves collected in 1965 and in 1966 within the Hubbard Brook Experimental Forest.

6. Compared to trees, the foliage of the herbaceous flora contained higher concentrations of potassium (2 to 4-fold greater) and probably of zinc. There appeared to be little change in chemical concentrations of the herbaceous flora during the summer. Species of spring herbs had consistently higher concentrations of P, Na and Cu, and consistently lower concentrations of Ca, Mg and Mn than did the same species collected in the summer.

7. These chemical analyses of plant tissues will be used to quantify the magnitude of nutrient pools in the Hubbard Brook Ecosystem and to evaluate the effects of these nutrient pools on input-output relationships and intrasystem nutrient cycling.

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